

Figure S1. The effect of donor plant growth environment (controlled conditions compared to field-grown plants) on vitality of microspores isolated from spikes. Experiment 2020/2021. **(a)** Spike at the optimal phase for ME induction; **(b, c)** Microspores from plants grown under controlled conditions; **(d, e)** Microspores from field-grown plants. Microspores stained with FDA (fluorescence of vital cells intensely green). Bar = 200 μm .

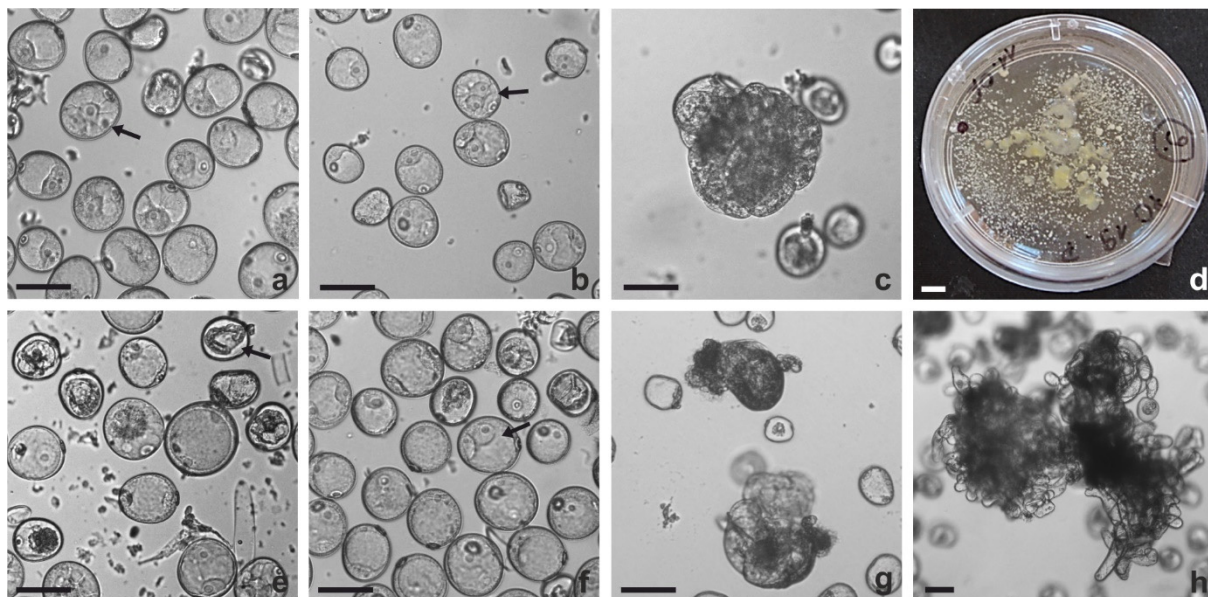


Figure S2. ME induction at 0, 14, 28 and 42d in suspension *in vitro* cultures of winter wheat (*Triticum aestivum* L.). **(a-d)** Properly developed embryo-like structures (ELS); **(e-h)** Suspension with low embryogenic potential to induce ELS with abnormalities.

(a) Microspores with star-like morphology (arrow points to SLS); **(b)** SLS after symmetric division (arrow); **(c)** Multicellular structure after 14d of culture; **(d)** Embryo-like structures (ELS) at the globular stage (0.5-1 mm in size) in co-culture with ovaries on Petri dish after 42 days of *in vitro* culture; **(e)** Microspore suspension after 14 days of culture (arrow points to dead microspore); **(f)** Microspores continuing gametophytic development in suspension; **(g)** Aborted multicellular structures; **(h)** Callus-like structure.

(a, b, e, f) Microspores suspensions on the day of isolation; **(c, g)** Microspore suspension after 14 days of culture; **(d)** Microspore suspension after 42 days of culture; **(h)** Microspore suspension after 28 days of culture; **(a-c, e-h)** Bar = 50 μ m; **(d)** Bar = 2 mm.

Table S1. Frequency of ME induction [ELS/spike] and green plant regeneration [GR/spike] in microspore cultures of winter wheat breeding lines of F1 and F4 generations. Experiment 2020/2021.

(*) Data for field grown plants; ELS – embryo like structures; GR – green regenerants

Wheat line	Experiment 2020/2021	
	ELS/spike	GR/spike
CH1	0	–
K20290	0.9	0
K393 F1	0	–
SM IHAR	0	–
MHR	0	–
PO19 F4	16*	0
PO20	2.0	0

(*) Data for field grown plants; ELS – embryo like structures; GR – green regenerants

Table S2. Frequency of ME induction [ELS/100 A] and green plant regeneration [GR/100 A] in anther cultures of winter wheat breeding lines of F1 and F4 generations. Experiment 202/2021.

Wheat line		Experiment 2020/2021			
		ELS/100A		GR/100A	
		C17 _{Fic}	C17 _{Ph}	C17 _{Fic}	C17 _{Ph}
CH1	F1	0.7*	0	0	–
K20290		0.8*	0	0	–
K393		2.0*	0	0	–
SM IHAR		3.2*	0	0.8	–
MHR		3.0*	0	2.0	–
PO19	F4	1.7*	0	0	–
PO20		2.8*	0.2*	0.3	0

(*) Data for field grown plants; C17Fic - C17 induction medium supplemented with 50 g/dm³ Ficoll 400; C17Ph - C17 induction medium supplemented with 2.5 g/dm³ Phytigel

Table S3. Frequency of ME induction [ELS/spike] and green plant regeneration [GR/spike] in isolated microspore cultures of winter wheat breeding lines of F1 and F4 generations. Experiment 2021/2022.

Wheat line		Experiment 2021/2022	
		ELS/spike	GR/spike
CH1		0	–
K20290		0	–
K393	F1	0.7	0
SM IHAR		0	–
MHR		0	–
PO19	F4	0.1-0.7*	0.2
PO20		0	–

(*) Data for field grown plants; ELS – embryo like structures; GR – green regenerants.

Table S4 Frequency of ME induction [ELS/100 A] and green plant regeneration [GR/100 A] in anther cultures of winter wheat breeding lines of F1 and F4 generations. Experiment 2021/2022.

Wheat line		Experiment 2021/2022											
		ELS/100A						GR/100A					
		C17 _{Fic}			C17 _{Ph}			C17 _{Fic}			C17 _{Ph}		
		10d	20d	30d	10d	20d	30d	10d	20d	30d	10d	20d	30d
CH1	F1	2.0	2.6	0.6	0.6	2.0	0	0	0	0	0	0	–
K20290		4.0	2.6	0	0.6	2.0	0	2.0	0	–	0	0	–
K393		0	0	0	0	0	0	–	–	–	–	–	–
SM IHAR		0.6	0	0	0	0	0	0.6	–	–	–	–	–
MHR		2.0	0	0	0.6	0	0	0	–	–	0	–	–
PO19	F4	3.4	0.6	0	0	0	0	0	0	–	–	–	–
PO20		2.0	0.6	0	0	0	0	0	0	–	–	–	–

C17Fic- C17 induction medium supplemented with 50 g/dm³ Ficoll 400; C17Ph - C17 induction medium solidified with 2.5 g/dm³ Phytigel; 10d, 20d, 30d – Low temperature tillers pre-treatment for 10, 20 or 30 days at 4°C; A – anthers; ELS – embryo like structures; GR – green regenerants